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MCCS-HCM STANDING OPERATING PROCEDURE 10 June 2002

PREPARATION OF PVA-FIXED SMEARS

1. INTRODUCTION:

Properly prepared slides of specimens fixed in polyvinyl alcohol (PVA) solution are essential to ensure good trichrome stains and to facilitate microscopic examination.

2. PRINCIPLE:

Polyvinyl alcohol is a water soluble plastic which when combined with Schaudin's fixative provides a good preservative fixative for protozoan trophozoites and cysts. The PVA fixative serves not only as a preservative but an adhesive also during the trichrome staining process.

3. SPECIMEN:

The specimen should be fixed in a ratio of at least 3 parts fixative to 1 part stool.

4. MATERIALS:

a. Frosted end glass slides.

b. Applicator sticks.

c. PVA fixative. (This is a mixture of polyvinyl alcohol and Schaudin's solution). The components are as follows:

Isopropanol	31.0%
Mercuric chloride	4.5%
Glacial acetic acid	5.0%
Glycerol	2.0% (Schaudin's solution)
Polyvinyl alcohol	5.0%
Purified water	52.5%

d. Slide warmer (temperature not critical).

5. PROCEDURE:

a. Preserved specimen (received in PVA vial).

(1) Label two frosted-end slides with the same accession number as the specimen.

(2) Shake the specimen by hand to mix the contents.

(3) Using the spoon attached to the inside top of the PVA vial, transfer a sample of the specimen to each of the two-labeled glass slides.

(4) Use an applicator stick to spread the specimen across the slide. It is desirable to have both thick and thin areas on the slide if possible.

(5) Place the slides on the slide warmer and allow them to dry overnight.

(6) Stain the slides with the trichrome procedure in use.

b. Fresh specimen (no preservative).

(1) Label two frosted end slides with the same accession number as the specimen.

(2) Place two drops of PVA fixative on each slide.

(3) Using applicator sticks, transfer a sample of the specimen to each slide, mixing the specimen with the PVA fixative on the slide. Strive for approximately a 1:3 specimen/PVA ratio. Spread the mixture across the slide, leaving both thick and thin areas.

(4) Place the slide on the slide warmer and allow them to dry overnight.

(5) Stain the slides with the trichrome procedure in use.

6. RESULTS:

Properly prepared slides should not be too thick or too thin. Remove high spots caused by insoluble particles with an applicator stick prior to staining to facilitate coverslipping.

7. QUALITY CONTROL:

a. Visually examine the PVA fixative used for fresh specimens, checking for precipitation or gelling. A thin haze of sediment at the bottom of the bottle is acceptable.

b. Prepare positive controls from known positive PVA vials and stained weekly. Record results.

8. SAFETY:

a. PVA fixative is a hazardous material.

(1) Avoid contact with skin and eyes. Should contact occur, flush with running water. If irritation develops, consult a physician. If ingested, dilute by drinking milk or water; contact a physician immediately.

(2) PVA fixative is corrosive and should be kept out of contact with metals. PVA fixative contains mercuric chloride, a mercury compound that is hazardous to the environment. All PVA vials should be discarded following established guidelines.

b. Unpreserved stools are potentially infectious. Wash hands frequently wear disposable gloves when working with specimens.

9. REFERENCES:

a. Beaver, P.C. and Jung, R.C., Clinical Parasitology. 9th ed., Philadelphia: Lea & Febiger, 1984.

b. Brooke, M.M. and Melvin, D.M., Laboratory Procedures for the Diagnosis of Intestinal Parasites. 3rd ed., U.S. Department of Health and Human Services, Centers for Disease Control, 1982.

c. Garcia, L. et al., Diagnostic Medical Parasitology 4th Ed.. New York: Elserier Science Publishing Co., 2001.